# SH2 domains: **A question of independence**

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**The three-dimensional structures of parts of two enzymes that contain tandem Src homology 2 (SH2) domains have recently been determined. The structures suggest how the SH2 domains function in concert to regulate enzymatic activity and localization.**

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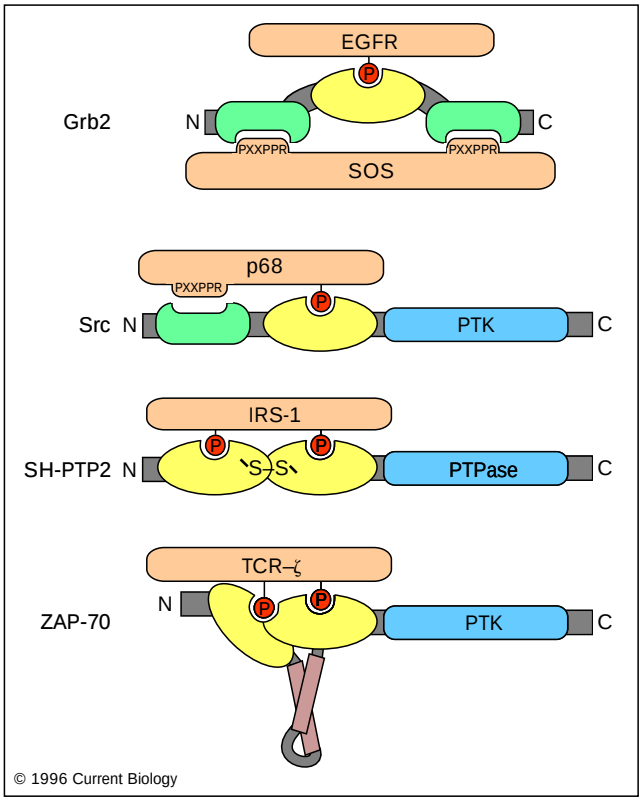
Phosphorylation is one of the most common currencies used in the business of signal transduction. The interplay between two classes of enzymes, kinases and phosphatases, controls the relative levels of the phosphorylated species that are essential for many signaling processes [1]. The substrates of these enzymes include tyrosine, serine, and threonine residues of proteins, as well as small molecules such as phosphatidylinositol. Two factors control the relative activities of kinases and phosphatases: localization of the enzymes and direct modulation of their catalytic activities [2]. The cellular signaling machinery, in response to stimuli, is able to regulate the level of phosphorylation and so control the degree of protein–protein association, required for intracellular signal transduction. Such associations are often mediated by relatively small protein subdomains that bind to specific proteins within the signaling cascade [3].

Whether they are found as isolated domains of proteins or attached to some catalytic machinery, Src homology 2 (SH2) and Src homology 3 (SH3) domains have become hallmarks of proteins involved in intracellular signal transduction (Fig. 1). SH2 domains bind tightly to phosphorylated tyrosine residues [4], and SH3 domains mediate protein–protein interactions through recognition of specific proline-rich sequences [5]. Structural biologists attacked these domains as soon as they were discovered, and the wealth of three-dimensional information now available includes ligand-bound as well as unbound structures for many SH2 and SH3 domains (see references within [6] for SH2 domains and within [5] for SH3 domains). SH2 and SH3 domains are often found within larger enzymes, but the complete structure of one of these enzymes has yet to be elucidated. Studies have focused instead on individual SH2 and SH3 domains. Recently, two structures have been determined in which two SH2 domains are structurally intertwined [6,7]. In each of these structures, the close associations between the two SH2 domains suggest how the phosphotyrosine-binding domains might act in concert to regulate enzymatic activity. Together with kinetic analyses, these structures provide a good opportunity for reassessing whether or not SH2 domains function independently of each other and of the catalytic domains to which they are often coupled.

Eck, Shoelson *et al.* [7] determined the three-dimensional structure of the two SH2 domains that constitute the amino-terminal regulatory domain of the protein tyrosine phosphatase SH-PTP2 (Fig. 1); this phosphatase has been implicated as a positive mediator of signal transduction downstream of several growth factor receptors [8]. The structure of the two SH2 domains (residues 1–225 of SH-PTP2), each bound to an identical phosphotyrosine-containing peptide, reveals that both domains have the same general fold found in other SH2 domains. The two sequences have 50 % identical amino acids, and their structures are nearly superimposable, including the extended 11 amino-acid phosphotyrosine-containing peptide ligand. A short stretch of four amino acids (residues 107–110) links the two domains, resulting in a considerable degree of shared surface area at the interface of the two domains. In an unexpected twist, there is a disulfide bond between Cys104 of the amino-terminal SH2 domain and Cys174 of the carboxy-terminal SH2 domain, and this bond is buried at the interface of the two SH2 domains. Intracellular proteins rarely have disulfide bonds because the cellular environment is reducing in nature, and Eck, Shoelson and colleagues took precautions to prevent oxidation during crystallization (the buffer contained 10 mM dithiothreitol); there is, as yet, no evidence that this disulfide bond is present within the cellular environment.

The tandem SH2 domains in its amino-terminal region have been shown to down-regulate the phosphatase activity of SH-PTP2 [9]. Truncation of the two SH2 domains yields an isolated catalytic domain that has 55-fold higher phosphatase activity than the full-length enzyme. Although it has been difficult to identify the cellular substrates of the phosphatase domain, cellular ligands of the SH2 domains have been identified and include the platelet-derived growth factor receptor (PDGFR) and the insulin receptor substrate-1 (IRS-1). Two of the 32 possible phosphotyrosine-containing peptides from IRS-1 are high-affinity ligands of the SH2 domains of SH-PTP2. The phosphorylated IRS-1 peptides containing residues pTyr1172 and pTyr1222 bind selectively to the amino-terminal and carboxy-terminal SH2 domains, respectively [9]. Alone or as an equimolar mixture, these peptides moderately stimulate the phosphatase activity of SH-PTP2, but full enzymatic activation (55-fold) is observed

**Figure 1**




Four proteins involved in intracellular signal transduction that contain SH2 or SH3 domains. The three-dimensional structure of Grb2 reveals how one SH2 domain and two SH3 domains may be independently arrayed to recognize their respective partner proteins [15]. The SH3 and SH2 domains of the Src-family protein tyrosine kinase, Lck, have a closer structural relationship [16]. This interdependence is probably shared by Src, in which both SH domains are required for autoinhibition and recognition of an associated phosphoprotein, p68. The regulatory domain of the protein tyrosine phosphatase SH-PTP2 contains two SH2 domains, and the SH2 domains found within ZAP-70 are also closely associated with each other (see text). The exact relationships between the regulatory domains shown here and their respective catalytic domains remain unknown, as structures of the full-length enzymes have not yet been determined.

only using two phosphotyrosine-containing peptides that are covalently linked through an appropriately chosen spacer element [10].

Enzymatic activation appears to be governed by the geometry of the ligands for the SH2 domains, and Shoelson and coworkers [10] have explored this phenomenon using two phosphotyrosine-containing peptides fused through a series of covalent linkers. Kinetic studies of SH-PTP2 reveal that phosphatase activation, through either removal or engagement of the SH2 domains, involves a change in the rate-determining step along the reaction coordinate pathway (T.J.W. and C.T. Walsh, unpublished observations). This change is such that engagement of the SH2 domains may initiate a rearrangement of the regulatory domain relative to the catalytic domain, resulting in phosphatase activation. The activated phosphatase domain of full-length SH-PTP2 is kinetically indistinguishable from the isolated phosphatase domain obtained by removal of the SH2 domains.

It appears that the two SH2 domains of SH-PTP2 have been fused, functionally as well as structurally, to form a larger enzyme-regulatory domain that is responsible for direct modulation of catalytic activity as well as protein localization. This regulatory role is dependent on engagement of both SH2 domains by phosphotyrosine-containing ligands of defined structure. This selective regulatory mechanism might allow SH-PTP2 to function in more than one capacity within multiple signaling cascades. For example, upon activation of the PDGFR, the amino-terminal SH2 domain of SH-PTP2 binds to a specific phosphotyrosine within the PDGFR [11]. SH-PTP2 is then phosphorylated on a tyrosine (pTyr542) located immediately carboxy-terminal to the phosphatase domain. This phosphotyrosine serves as a binding site for the adaptor protein Grb2, which is recruited to the membrane and activates the Ras signaling pathway [11]. Within this signaling cascade, SH-PTP2 functions as an adaptor, and engagement of only the amino-terminal SH2 domain might allow SH-PTP2 to fulfil its signaling role without stimulation of phosphatase activity. In other circumstances, engagement of both SH2 domains by a protein such as IRS-1 may liberate the repressed catalytic machinery and allow SHPTP2 to participate in signaling pathways through dephosphorylation of phosphotyrosine-containing substrates.

A similar regulatory domain composed of two SH2 domains has been found within the T-cell tyrosine kinase, zeta-associated protein (ZAP-70). Upon T-cell activation, several subunits of the T-cell receptor become tyrosine-phosphorylated and ZAP-70, an essential component of this signaling cascade, associates with the activated receptor through its SH2 domains (Fig. 1) [12]. A group from Ariad Pharmaceuticals [6] has determined the three-dimensional structure of the ZAP-70 amino-terminal regulatory domain (residues 1–259) in a complex with a doubly phosphorylated 19-residue phosphotyrosine-containing peptide from the ζ-chain of the T-cell receptor. The SH2 domains display the now-familiar fold typical of the family and are separated from each other by a 65-residue stretch of two α-helices that form a coiled coil. The carboxy-terminal SH2 domain binds to its cognate ligand through interactions that are similar to those observed in SH-PTP2–ligand and other SH2 domain–ligand complexes: two pockets along the surface of the SH2 domain are occupied by the phosphotyrosine side chain and the leucine side chain in the (pTyr + 3) position. For the other SH2 domain a departure from the conventional binding motif is

found: within the amino-terminal SH2 domain, a hydrophobic pocket also accommodates the (pTyr + 3) leucine, but the phosphotyrosine-binding pocket is sandwiched at the interface of the two SH2 domains (Fig. 1). Stabilizing contacts to the phosphotyrosine side chain are donated by both the amino-terminal and the carboxy-terminal SH2 domains, providing a three-dimensional explanation for the experimental observation that the individual ZAP-70 SH2 domains do not bind to phosphotyrosine-containing peptide ligands [13].

The authors suggest [6] that the organization of the liganded SH2 domains might exert allosteric effects on the catalytic domain of ZAP-70. In support of this theory, the closely related kinase Syk is stimulated upon engagement of its SH2 domains by phosphotyrosine-containing peptide ligands [14]. Unlike the covalently fused SH2 domains from SH-PTP2, the two SH2 domains from ZAP-70 may not dimerize in the absence of phosphotyrosine-containing ligands. The X-ray structure reveals that the two SH2 domains share a modest 200 $Å^2$ of surface area with each other, whereas the surface area buried between regulatory domain and the extended peptide ligand amounts to 1300 $Å^2$, in a significantly stabilizing interaction. Assembly of the regulatory SH2 domains is assisted by the inter-domain coiled coil as well as by contacts between the doubly phosphorylated peptide and the SH2 domains. The tandem SH2 domains within SH-PTP2, and those within ZAP-70, are sandwiched together and provide a sensitive mechanism for the modulation of catalytic activity upon binding to phosphotyrosine-containing peptides.

The structures of the SH-PTP2 and ZAP-70 regulatory domains provide the first three-dimensional views of how Src homology domains might regulate the enzymatic activities of catalytic domains with which they are associated [6,7]. Within the regulatory domains, single SH2 domains are not as independent as one might have predicted on the basis of previous structures. But the picture is far from complete in the absence of structural information relating these regulatory domains to their respective catalytic domains. Structural biology complements biochemical and molecular genetic techniques as a powerful means of elucidating mechanisms of signal transduction, and an atomic-resolution understanding of the regulation of these enzymes will aid efforts to manipulate signaling cascades for therapeutic purposes. ZAP-70 appears to be a unique component of the T-cell activation machinery, and molecules that interfere with the association between ZAP-70 and the ζ-chain of the T-cell receptor may prove to be selective immunosuppressive agents. The SH2 domains within SH-PTP2 and ZAP-70 modulate the localization and enzymatic activity of the catalytic domains to which they are attached, and the structures of these regulatory domains provide two distinct models of how Nature might use more than one modular protein subdomain to govern cellular signaling.

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